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Long non-coding RNA *LUCAT1* promotes tumorigenesis by controlling ubiquitination and stability of DNA methyltransferase1 in esophageal squamous cell carcinoma

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Long non-coding RNA *LUCAT1* promotes tumorigenesis by controlling ubiquitination and stability of DNA methyltransferase1 in esophageal squamous cell carcinoma

Directed by Professor Sang Kil Lee

The Doctoral Dissertation
submitted to the Department of Medical Science,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy

Jung-ho Yoon

December 2017

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학위과정을 마치며 감사의 말씀을 전하고자 합니다.
공백기간이 있고 다시 시작한 학업이었기 때문에 새로운 연구분야에 도전해 보고 싶었고, 학위과정 동안 연구한 Long non-coding RNA 는 저에게는 도전이자 기회였습니다. 먼저, 이런 좋은 연구주제로 연구 할 수 있게 기회를 주신 지도교수님 이상길 교수님께 감사 드립니다. 교수님의 따뜻한 지도와 가르침으로 학위과정을 무사히 마칠 수 있었습니다. 다시 한번 교수님께 지도 받을 수 있었고, 연구 할 수 있게 기회를 주신 것에 대해 큰 감사 드립니다. 진료 및 연구활동으로 바쁘신 와중에도 학위논문을 지도해주신 심사위원 교수님들, 정재호 교수님, 윤영훈 교수님, 조병철 교수님 그리고 늘 가까이서 관심과 지도로 격려를 해주신 이용찬 교수님께 감사 드립니다.

학위과정 동안 어렵고 힘든 일이 있을 때마다 도움을 주었던 분들에게도 감사의 인사를 이 글을 통해서 전합니다. 실험을 진행하면서 많은 도움을 주시고, 한층 더 질 좋은 연구가 될 수 있도록 도와주신 한양대학교 남진우 교수님과 유보현 선생님께 감사 드립니다. 멘토가 되어주시는 김택중 교수님 항상 감사드립니다.

밤늦게까지 같이 실험하며 페이스메이커가 되어 준 나금이, 진심으로 고맙습니다. 또한 실험적으로나 내적으로 항상 기댈 곳이 되어 주던 윤희형, 실험도 많이 도와주고, 벗이 되어주던 종주형, 아직 학생이라는 이유로 모임이 있을 때 마다 배려해준 영수형, 욱진이형, 주호형 등 많은 선배님들에게 감사의 말 전합니다.

자주 만나지는 못하지만 멀리서 응원해주는 용재, 주홍이, 힘들 때 마다 힘이 되어 준 유리 그리고 실험실 선배로서 많이 부족하지만 잘 따라주고 도움 주고 있는 효주, 많은 디스커션을 해주신 송준규 선생님, 황보람 선생님, 이소담 선생님에게도 고마움을 전합니다.

언제나 제 편이 되어주고, 부족한 아들 자랑스럽게 여겨주시는 사랑하고 존경하는 아버지, 어머니께 항상 감사 드리고 사랑한다는 말, 표현은 못하지만 이 글을 통해 올립니다. 앞으로도 항상 자랑스러운 아들, 또 아버지 같은 아빠가 되기 위해 노력하고 실천하겠습니다. 물심양면으로 많은 도움을 주시고, 미래를 기대해주시는 어머님, 아버님에게도 진심으로 감사 드립니다. 내색은 안 하지만 형을 항상 응원해주는 공부하느라 힘든 동생 준호에게도 늘 미안하고 고맙다는 말을 전합니다.

마지막으로 힘들어 할 때 마다 항상 옆에서 웃어주고, 힘이 되어주는 사랑하는 아내 윤현정과 아직까지 모든 것이 서툰 초보 엄마, 아빠 옆에서 건강하게 잘 자라주고 있는 내 딸 다현이에게 감사의 말을 전합니다.

2017 년 12 월

윤 정 호 배상

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ABSTRACT

Long non-coding RNA *LUCAT1* promotes tumorigenesis by controlling ubiquitination and stability of DNA methyltransferase1 in esophageal squamous cell carcinoma

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(Directed by Professor Sang Kil Lee)

Esophageal squamous cell carcinoma (ESCC) is a fatal malignancy whose pathophysiology is not well known. Recently, a considerable number of long non-coding RNAs (lncRNA) have been reported to be involved in various cancers. In this study, we validated the mechanism of *LUCAT1* in ESCC. We used next-generation transcriptome sequencing (RNA-seq) to analyse transcriptomes using thirteen paired adjacent normal tissues and ESCC tissues. Among the lncRNAs, *LUCAT1* was

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significantly overexpressed in ESCC compared to adjacent normal tissues (p <0.001). *LUCAT1* was significantly upregulated in ESCC cell lines and cancer tissue of patients with ESCC compared to normal cells and adjacent normal tissue, respectively (p <0.001). The expression of *LUCAT1* in normal tissues adjacent to cancer of ESCC patients was significantly high compared to that in the tissue of normal patients (p <0.001). *LUCAT1* blocked the apoptosis and induced the migration and invasion of KYSE-30 cell lines and HCE-4 cell lines. *LUCAT1* siRNAs up-regulated the tumor suppressor genes, including GADD45G, GAS1, HPGD, and SFRP2 by reducing the methylation of these genes. *LUCAT1* siRNAs reduced the protein level of DNA methyltransferase 1 (DNMT1) without interfering with transcription. *LUCAT1* controlled the ubiquitination and degradation of DNMT1 in an E3 ubiquitin-protein ligase UHRF1-dependent manner. Patients with high *LUCAT1* had a significantly lower survival rate than patients with low *LUCAT1*. In conclusion, our results suggest that the lncRNA *LUCAT1* regulates the stability of DNMT1 and inhibits the expression of tumor suppressors through DNA methylation, leading to the proliferation and metastasis of ESCC. We found that *LUCAT1* could be a drug and biomarker target for ESCC in this study.

Key words: long non-coding RNA, *LUCAT1*, DNA methyltransferase 1, esophageal squamous cell carcinoma

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Ⅰ. INTRODUCTION

Esophageal cancer is the eighth most common cancer and the sixth leading cause of cancer related mortality.[1] The two main types of esophageal cancer are squamous cell carcinoma (ESCC) and adenocarcinoma. ESCC is linked to heavy smoking and alcohol consumption.[2] Despite recent advances in clinical practice, the prognosis of ESCC patients is poor, as until now, there have not been any targets for drug development in the major pathways involved in the development of ESCC. Therefore, it is urgent to

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research new key routes to ESCC and find targets to treat ESCC.

Recently, studies using ESCC cells and clinical tissues have shown that the suppression of tumor suppressor expression by DNA methylation is important for the early development and progression of esophageal cancer.[3] However, the detailed mechanisms leading to ESCC have yet to be fully elucidated, and it is important to develop new potential biomarkers for early diagnosis and new treatments.

Long non-coding RNAs (lncRNAs) are functionally defined as transcripts >200 nt in length with no protein-coding potential, and many are noted for their role in controlling the major factors driving carcinogenesis in various cancers.[4] Although the mechanisms of lncRNAs have not been well documented, they can be separated based on their influence on chromatin state and methylation, the stability of proteins and complexes, or by acting as a sponge for miRNA inhibition.[5] LncRNA has recently been noted for its role in controlling many of the major factors driving the post-transcriptional and epigenetic mechanisms. Many cancer studies are underway, but the function of lncRNA in esophageal cancer is not well known. Recently, the interactions between lncRNA and DNA methylation have been considered important for cancer biology. [6]

Epigenetic changes caused by DNA methylation are important for the onset and progression of cancer.[7] Promoter methylation of the tumor suppressor results in

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transcriptional silencing and loss of gene function.[8] DNA methylation is performed by three DNA methyltransferases: DNMT1, DNMT3a, and DNMT3b.[9] DNMT1 is primarily involved in methylation maintenance, and hemisomylated cytosines can be methylated on double-stranded DNA molecules, which may be involved in the methylation of the strand.[9] However, DNMT3a and DNMT3b play an important role in de novo methylation. Methylation can be performed on unmethylated double-stranded DNA. DNA methylation is generally associated with gene suppression, and DNA demethylation is usually associated with gene activation. The control of DNA methylation by lncRNA can be an important mechanism in the development and regulation of gene expression in disease. Recently, the relevance of lncRNAs to proliferative factors has been widely reported.[10-12]

Next-generation transcriptome sequencing (RNA-seq) provides a way to describe all sets of transcription errors in diseases, including lncRNAs and protein-coding genes. Using RNA-seq to analyze various cancer tissues, functional lncRNAs in various cancers have recently been defined.[13-16] However, functional lncRNAs in ESCC have yet to be identified. Here, we perform RNA-seq to investigate the expression levels of lncRNAs in 13 paired ESCC tissues.

The LncRNA *LUCAT1* was first reported to be involved in lung cancer caused by smoking.[17] It is well known that smoking is the most potent cause of ESCC, so it can

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be assumed that *LUCAT1* may be involved in the carcinogenesis of ESCC. In this study, we investigate the role of *LUCAT1* in the proliferation and invasion of ESCC and find that *LUCAT1* plays an important role in the progression of ESCC and that the expression of *LUCAT1* is a predictor of survival. We show that *LUCAT1* interacts with DNMT1. In addition, we find that DNMT1 stability, which can regulate DNA methylation, is associated with *LUCAT1* expression levels. Thus, *LUCAT1* plays an important role in the survival and proliferation of ESCC.

Ⅱ. MATERIALS AND METHODS

1. Cell lines and cell culture

KYSE-30 ESCC cells were cultured in RPMI 1640 medium (Thermo Scientific, Rockford, IL, USA) supplemented with Ham's F12 medium, 10% fetal bovine serum (FBS; Thermo Scientific), and 1% penicillin/streptomycin (Thermo Scientific). TE-2, HCE-4, and HCE-7 cells were cultured in Dulbecco's modified Eagle's medium (Thermo Scientific) supplemented with 10% FBS and 1% penicillin/streptomycin. HET-1a immortalized normal esophageal cells were cultured in BEGM medium supplemented with a BEGM Bullet Kit (Lonza, Walkersville, MD, USA). The growth medium was also supplemented with 10% FBA and 1% penicillin/streptomycin. All

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cells were incubated in a humidified atmosphere with 5% CO2 at 37℃. TE-2, TE-3, HCE-4, and HCE-7 cells were kindly provided by Dr. KH Kim (Yonsei University Medical School, Seoul, Korea), and the other cell lines were obtained from American Type Culture Collection (ATCC, Rockville, MD, USA).

2. ESCC patients and tissue sampling

Thirty-eight patients with ESCC were enrolled for this study. The clinicopathologic findings and tissue were collected prospectively and summarized in Table 1. All study participants provided informed consent, and the study design was approved by the Public Institutional Bioethics Committee designated by the Ministry of Health and Welfare (South Korea) (No. 4-2014-0775). Tissue samples were collected by endoscopic biopsy from cancer and adjacent normal appearing mucosa. The normal tissues from normal patients were obtained from another clinical study on non-erosive reflux disease. The collected tissues were immediately transferred to RNAlater solution (Ambion), delivered to the laboratory, and then stored in a deep refrigerator until analysis.

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Table 1.Clinicopathologic characteristics of enrolled patients

Characteristics	N = 38
*Age, yr (range)	68 (52 - 91)
Sex	
Male	31 (82%)
Female	7 (18%)
Stage	
I	6 (16%)
II	6 (16%)
III	21 (55%)
IV	5 (13%)
Differentiation	
ESCC, WD	3 (8%)
ESCC, MD	26 (68%)
ESCC, PD	2 (5%)
ESCC, uncertain	7 (19%)
Initial treatment	
Surgery	11 (29%)
Chemoradiotherapy	16 (42%)
Chemotherapy only	2 (5%)
Radiotherapy only	1 (3%)
None	8 (21%)

* Data represented as median value

ESCC, esophageal squamous cell carcinoma; WD, well differentiated; MD, moderately differentiated; PD, poorly differentiated

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3. Total RNA extraction, reverse transcription and quantitative real-time PCR

Total RNA extraction from ESCC cells and tissues was performed using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). RNA was quantified using a Nanodrop (ND-100; Nanodrop Technologies lnc., Wilmington, DE, USA), and purity was determined based on the 260/280 nm ratio and by analysis on 1% agarose gels. cDNA synthesis was performed using 2.0 μg of total RNA with Superscript II (Invitrogen). The relative expression of *LUCAT1* was determined and analyzed by quantitative real-time PCR using a Light Cycler 480 Real-Time PCR machine and iQ SYBR Green Supermix (Applied Biosystems Inc., Carlsbad, CA, USA). The Ct value of the sample was normalized to the U6 or GAPDH expression, and the 2-ΔΔCt value was calculated. Primers used for qRT-PCR are shown in Table 2.

Table 2. Primer sequences for qRT-PCR

Gene	Direction	Sequence (5' to 3')
LUCAT1	Forward	5'- GCTCGGATTGCCTTAGACAG -3'
	Reverse	5'- GGGTGAGCTTCTTGTGAGGA -3'
MEG3	Forward	5'- CAGCCAAGCTTCTTGAAAGG -3'
	Reverse	5'- TTCCACGGAGTAGAGCGAGT -3'
ZFPM-AS1	Forward	5'- AAGAGGTCAGGAGGCACCTT -3'
	Reverse	5'- AGGCAGGAGAATTGCTTGAA -3'
RP5-1112F19.2	Forward	5'- GTGAAACCCCGTCTCTACCA -3'
	Reverse	5'- CAAGCAATTCTCCTGCCTTC -3'

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DNMT1	Forward	5'- ACGACCCTGACCTCAAATAT -3'
	Reverse	5'- CCATTAACACCACCTTCAAGA -3'
GADD45G	Forward	5'-GGTGAGTACAGTCCCGTCC-3'
	Reverse	5'-GCAAAACAGGCTGAGCTTCT-3'
SFRP2	Forward	5'-AGTGGTGTTTGGTGGAGGAG-3'
	Reverse	5'-TGCATCCTGGTTTTCCTTTC-3'
GAS1	Forward	5'-GAAACTCCCAACTCGTCTGC-3'
	Reverse	5'-ACCTTCCCTTTCGAGTCCAG-3'
HPGD	Forward	5'-TAGCCATTCTGATCGCTGTG-3'
	Reverse	5'-AGACACATGGCCAACAAACA-3'
U6	Forward	5'-CTCGCTTCGGCAGCACA-3'
	Reverse	5'-AACGCTTCAGGAATTTGCGT-3'
GAPDH	Forward	5'-CCGGGAAACTGTGGCGTGATGG-3'
	Reverse	5'-AGGTGGAGGAGTGGGTGTCGCTGTT-3'

4. Small interfering RNA (siRNA) transfection

For transfection, all cells are counted in 6 wells ($3X10^5$) and incubated in a 37 ° C incubator. After 24 hours, siRNA *LUCAT1* and RNAi negative control (siCT; Invitrogen, Carlsbad, Calif., USA) were transfected according to the protocol of Lipofectamine 2000 using Lipofectamine 2000 reagent (Invitrogen). The sequences of target siRNAs for *LUCAT1* are listed in Table 3.

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Table 3. siRNAs targeting lncRNA *LUCAT1*

Gene	Direction	Sequence (5' to 3')
Si LUCAT1_1	Sense	5'- CAG AAG AUG UCA GAA GAU AAG GAU U-3'
	Antisense	5'-A AUC CUU AUC UUC UGA CAU CUU CUG-3'
Si LUCAT1_2	Sense	5'-GCACAGAUAAAUUUCUCUUACUGUA-3'
	Antisense	5'-UACAGUAAGAGAAAUUUAUCUGUGC-3'

5. *LUCAT1* overexpression plasmid construction

LUCAT1 cDNA was amplified using a PCR system (Roche Applied Science). To insert the cDNA into the pcDNA3.1 (+) expression vector, *LUCAT1*_NHel_F (acccaagctggctagc CAATGCCCAGACCTCCAG) and *LUCAT1*_Xbal_R (aaacgggccctctaga TTGACTGCAAGAGCTTGAAG) were used as cloning primers.

The pcDNA3.1 (+) expression vector was purchased from Addgene. KYSE-30 cells were transfected with 1 μg of pcDNA3.1-LUCAT1 for 24 h using Lipofectamine 2000 (Invitrogen).

6. Cell proliferation analysis

MTS assays (Promega, Madison, WI, USA) were used to measure cell proliferation in 96-well plates. Plates were incubated in a dark room for 1 h, and enzyme-linked

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immunosorbent assays (ELISAs) were performed. Briefly, ESCC cells were transfected with 50 nM siLUCAT1 or siCT, and cell proliferation was measured from 0 to 72 h. For rescue experiments, cells were transfected with pcDNA-LUCAT1 48 h after siRNA transfection, and cell proliferation was assessed.

7. Apoptosis analysis

KYSE-30 cells were transfected with *LUCAT1* siRNA or siCT, and the cell pellet was recovered after 48 h. The cell pellet was resuspended in 1× binding buffer (BD Bioscience, San Jose, CA, USA) and phosphate-buffered saline (PBS). Cells were stained with propidium iodide and fluorescein isothiocyanate (FITC) annexin V using a FITC-Annexin V kit (BD Bioscience). For analysis by flow cytometry using a FACSverse instrument (BD Biosciences), stained cells were incubated at for 15 min. Data were analyzed using Flow Jo software (Treestar, Ashland, OR, USA). Three experiments were conducted for each assay.

8. Invasion assay and Migration assay

To perform the invasion assay, two siLUCAT1s were transfected into KYSE-30 cells, and the cells were then reseeded in Matrigel Invasion Chambers (BD Biosciences) in a 24-well culture plate. The bottom chamber was filled with medium containing 10% FBS. After 24 h, the non-invading cells were removed from the insertion chamber

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using a cotton swab. The lower side of the upper chamber was fixed and stained with Diff-Quik stain (Dade Behring Inc., Newark, DE, USA). Invading cells were visualized using a virtual microscope (BX51; Olympus, Tokyo, Japan) in five random fields, counted, and averaged.

For migration analysis, KYSE-30 cells (2×10^5) were transfected with siLUCAT1s and siCT. The cells were grown for 24 h, and a wound was then generated using a P200 pipette tip. A virtual microscope (BX51; Olympus) was used to measure the wound width at 0 and 24 h. Image analysis was performed using Image J software (NIH). The experiment was performed three times.

9. Western blot

Whole lysates were used with 1× RIPA buffer (Cell Signaling Technology, Danvers, MA, USA) containing protease inhibitor (GenDEPOT, Barker, TX, USA). Protein was separated using sodium dodecyl sulfate-polyacrylamide gels and transferred to polyvinylidene fluoride membranes (Millipore, Darmstadt, Germany). After blocking for 30 min at room temperature with 3% bovine serum albumin (Thermo Scientific), the membranes were incubated with primary antibodies, followed by incubation with horseradish peroxidase-conjugated goat anti-mouse and goat anti-rabbit secondary antibodies (GenDEPOT). For western blot analysis, the following antibodies were used:

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anti-mono- and polyubiquitinylated monoclonal antibodies (Enzo, USA), anti-DNA methyltransferase 1 (DNMT1 Abcam, USA), anti-zinc finger E-box binding homeobox 1 (ZEB1 Cell Signaling Technology), anti-E-cadherin (BD Biosciences), anti-N-cadherin (BD Biosciences), anti-Bcl-2 (Cell Signaling Technology), anti-Bcl-xl (Cell Signaling Technology), anti-p53 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), and anti-β-actin (Thermo Scientific). The membranes were then reacted with ECL solution (GenDEPOT) and exposed to an X-ray film processor (CP1000; AGFA, Greenville, SC, USA) or an Image Quant LAS 4000 (GE Healthcare, Piscataway, NJ, USA).

10. RNA immunoprecipitation (RIP)

For immunoprecipitation of endogenous RNA-protein complexes, cells were lysed with IP buffer (Thermo Fisher Scientific, USA) and resuspended in RIP buffer (Abcam) with RNase inhibitor (GenDEPOT) and protease inhibitor (GenDEPOT). For shearing of chromatin, we used 20 cycles of shearing under cooling conditions, with 15s on and 30s off for each cycle (170–190 W) and water bath sonication. After sonication, the antibodies were added to the supernatant obtained by centrifugation and then incubated overnight at 4°C with a rotator. After incubation, 20 μL of Magna Chip protein magnetic beads (Millipore) was added, and samples were reacted on a rotator at 4℃

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for 1 h. After washing twice with RIP buffer, samples were dissolved with TRIzol reagent or RIPA.

11. Methylation specific PCR (MS-PCR)

Genomic DNA was extracted from cells using the DNeasy Blood & Tissue kit (Qiagen, Valencia, CA, USA). DNA bisulfate conversion was performed with the EZ DNA Methylation-Gold Kit™ (Zymo Research, Irvine, CA, USA) followed by MS-PCR. The MS-PCR primers used for target genes are shown in Table 4.

Table 4. Primer sequences for MS-PCR

Gene	**Direction**	**Sequence (5' to 3')**
GADD45G	Forward	5'- TTTAGGAAGGTTTAGGTTAGGACGT -3'
Methylation	Reverse	5'- GTTTTAAAAAAACAACGCAATCG -3'
GADD45G	Forward	5'- AGTAGTAGTAGTAAGATGGAGGCGA -3'
Unmethylation	Reverse	5'- ATTTTAAAAAAACAACACAATCAAC -3'
GAS1	Forward	5'- AAGAGGTCAGGAGGCACCTT -3'
Methylation	Reverse	5'- AAAACCTACCCTATAAACCTAAACG -3'
GAS1	Forward	5'- AGTAGTAGTAGTAAGATGGAGGTGA -3'
Unmethylation	Reverse	5'-AACCTACCCTATAAACCTAAACACA -3'
SFRP2_1	Forward	5'- GTTTGTCGGAGTTATTAGGATGTC -3'
Methylation	Reverse	5'- CTAATAATCACCTCGATAAAACGAT -3'
SFRP2_1	Forward	5'-TTGTTGGAGTTATTAGGATGTTGG-3'
Unmethylation	Reverse	5'-CTAATAATCACCTCAATAAAACAAT-3'
SFRP2_2	Forward	5'-GGAAACGGTCGTATTTAAGTATGTC-3'

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Methylation	Reverse	5'-CACCAAAAAATTCCTATACTCGCT-3'
SFRP2_2	Forward	5'-GGGAAATGGTTGTATTTAAGTATGTT-3'
Unmethylation	Reverse	5'-ACACCAAAAAATTCCTATACTCACT-3'
HPGD	Forward	5'-GATTTTTTATAATTGTTTTTTTCGT-3'
Methylation	Reverse	5'-ACCATCCATAAAACTTTTACACG-3'
HPGD	Forward	5'-ATTTTTTATAATTGTTTTTTTTGT-3'
Unmethylation	Reverse	5'-CAACCATCCATAAAACTTTTACACAC-3'

12. RNA-sequencing followed by next-generation sequencing (NGS)

RNA from 13 paired adjacent normal tissues and ESCC tissues was extracted using TRIZOL reagent (Invitrogen) for RNA extraction. Illumina Truseq stranded and unstranded mRNA library prep kits (Illumina, USA) were used for deep sequencing library preparation according to the manufacturer's protocol. Libraries were sequenced in paired-end format to a length of 101 bp using the HiSeq 2000 platform (Macrogen Corporation, Republic of Korea).

13. Expression profiling

All RNA-seq data ENCODE, TCGA, and GTExRPDs were used to quantify gene expression values based on annotations using feature Counts version 1.5.1 with parameters -t exon -g gene_id –min Overlap "average length of reads divided by 2" -s

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2. The -T option was specified only for paired-end reads with -p -B -C.

14. Statistical analysis

All data were analyzed for continuous and categorical variables and presented as means ± standard error of the mean (SEM). Statistical tests included the t-test, $\chi 2$ test, and Fisher's exact test. The degree of expression of *LUCAT1* in ESCC was categorized as low or high based on the median of *LUCAT1* expression. Continuous variables were presented as means ± standard deviation. Categorical variables were presented as numbers with percentages. Descriptive statistics were used to characterize the baseline clinicopathologic characteristics of patients. Survival curves were estimated according to the Kaplan–Meier method, and the differences according to *LUCAT1* expression were examined using the log-rank test. Additionally, Fisher's exact test, Student's t-test, and the Mann–Whitney U test were used to compare *LUCAT1* expression with the clinical T stage, N stage, M stage, and overall stage of the ESCC patients. However, there was no statistically significant difference (data not shown). A value of $p < 0.05$ was considered statistically significant. All statistical procedures were conducted using the statistical software SPSS for Windows (version 18.0; SPSS Inc., Chicago, IL, USA).

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Ⅲ. RESULTS

1. ESCC related lncRNA expression analysis from RNA-seq data

To determine the expression of lncRNAs in esophageal carcinoma, RNA-seq was performed using RNA of three pairs of esophageal cancer patients (Fig. 1a). As a result, we selected four annotated lncRNAs that were highly expressed in cancer tissues compared with adjacent tissues (Table 5). Among lncRNAs, *ZFPM2-AS1, MEG3, RP5-1112F19.2,* and *LUCAT1* were significantly overexpressed in ESCC compared to adjacent normal tissues (p<0.001) (Fig. 1b). *LUCAT1* was located at chr5:90597115-90610219 (Fig. 1c). In addition, RNA-seq analysis of 10 patients with esophageal cancer was performed. In these RNA-seq results, the adjusted *p*-value of *LUCAT1* was not valid. However, the RNA-seq results of all 13 patient RNAs showed valid values (Table 6). The RNA-seq analysis of all 13 patients with esophageal cancer confirmed the expression of *LUCAT1* in esophageal cancer. Previous reports on the RNA-seq data[18] and TCGA data of 15 Chinese patients with esophageal cancer also showed that *LUCAT1* expression was greater in cancer. *LUCAT1* was more highly expressed in cancer compared to normal tissue samples (GTEx normal 338 non-paired samples) with ESCC (TCGA tumor 95 non-paired samples) and ESAD (TCGA tumor 89 non-paired samples) in TCGA data. In addition, in HNSC (43 paired samples), LUSC (51

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paired samples), and LUAD (58 paired samples), *LUCAT1* was also more highly expressed than in normal samples in TCGA data (Fig. 2).

Table 5. Highly expressed lncRNAs

gene_id	locus	fold Change	log2_fold Change	*p*-value	adjusted *p*-value
ZFPM2-AS1 (lncRNA)	chr8:106791753-107072752	288.783352	8.173843765	1.36E-08	0.0001133
MEG3 (lncRNA)	chr14:101245746-101327354	23.3410585	4.544798083	0.000055	0.0758876
RP5-1112F19.2 (lncRNA)	chr20:50448335-50479451	9.73462487	3.283125386	4.05E-05	0.0758876
LUCAT1 (lncRNA)	chr5:90598115-90610219	16.1155246	4.010379249	0.00028	0.211573

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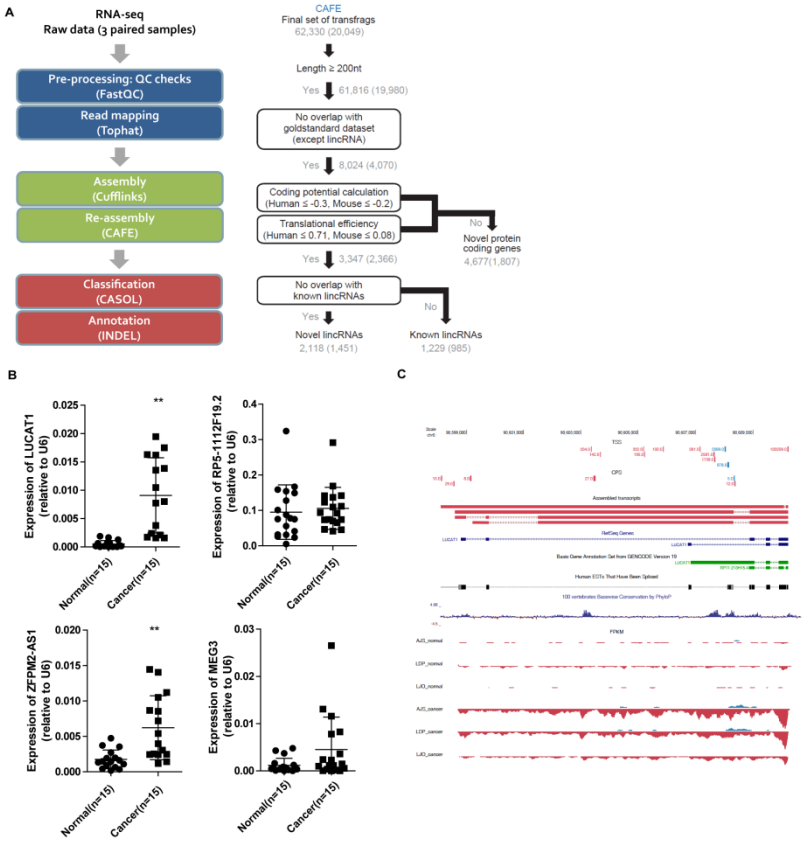

Figure 1. Genome wide analysis of lncRNA expression by RNA-seq. Schematic representation of RNA-seq analysis workflow (a). LncRNA levels were measured by SYBR qRT-PCR and calculated by 2-ΔΔCT (* $p \leq 0.05$; ** $p \leq 0.01$). Expression of *LUCAT1* was higher than that of *RP5-1112F19.2*, *ZFPM-AS1*, and *MEG3* in the ESCC tissues than in the adjacent normal mucosa (n=15) (b). Location of lncRNA *LUCAT1* and RNA-seq data reads were visualized using University of California, Santa Cruz (UCSC) Genome Browser (c).

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Table 6. Highly expressed *LUCAT1*

Gene_id	Locus	Fold Change	Log2_fold Change	*p*-value	Adjusted *p*-value
LUCAT1 (lncRNA)	chr5: 90598115-90610219	11.8702	3.5692	6.00E-15	1.30E-13

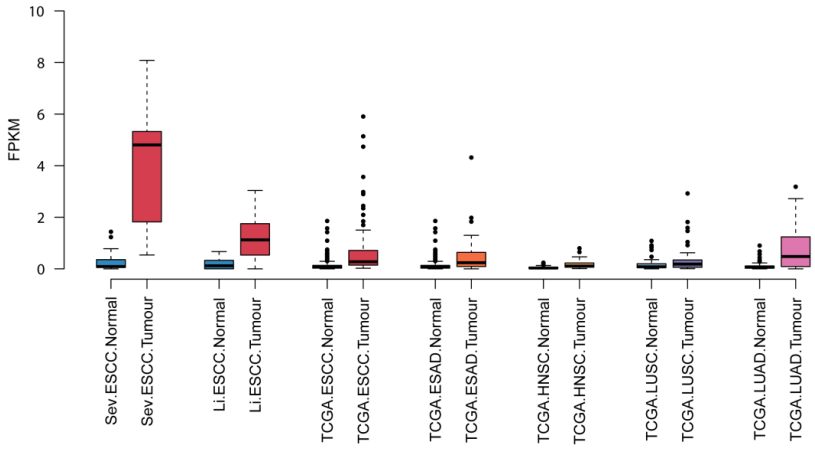

Figure 2. Expression of *LUCAT1* in RNA-seq data and TCGA data. The first bar (Sev) shows the expression level of LUCAT1 in the RNA-seq data (13 paired samples), and the next bar (Li) shows the expression level of *LUCAT1* using RNA-seq data (15 paired samples) reported in China.[18] The expression level of *LUCAT1* was also expressed using TCGA data. In order, the *p*-values are 0.0007324, 0.0004272, 2.2e-16, 1.043e-13, 4.101-e05, 0.01794, and 2.713e-08.

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2. *LUCAT1* is highly overexpressed in ESCC cell lines and cancer tissues

LUCAT1 was significantly elevated in the five ESCC cell lines compared to normal esophageal HET-1A cells (Fig. 3a). The expression of *LUCAT1* in cancer tissues and adjacent normal tissues from 38 patients with ESCC and 20 normal tissues from normal patients was measured by qRT-PCR. The expression of *LUCAT1* was significantly higher in cancer tissues compared with adjacent normal tissues of patients with ESCC (p <0.001) (Fig. 3b). The mean Ct value of *LUCAT1* was about 14 times higher in cancer tissues than in adjacent normal tissues. The levels of *LUCAT1* of normal tissues of 20 normal patients were significantly lower than those of adjacent normal tissues of patients with ESCC (p<0.001). Furthermore, the Ct value of *LUCAT1* was about 318 times higher in adjacent normal tissues compared to normal tissues of normal patients. According to this finding, *LUCAT1* might play a role in the early stages of ESCC carcinogenesis. The incensement of *LUCAT1* in patients with ESCC was also confirmed in the TCGA database (Fig. 2).

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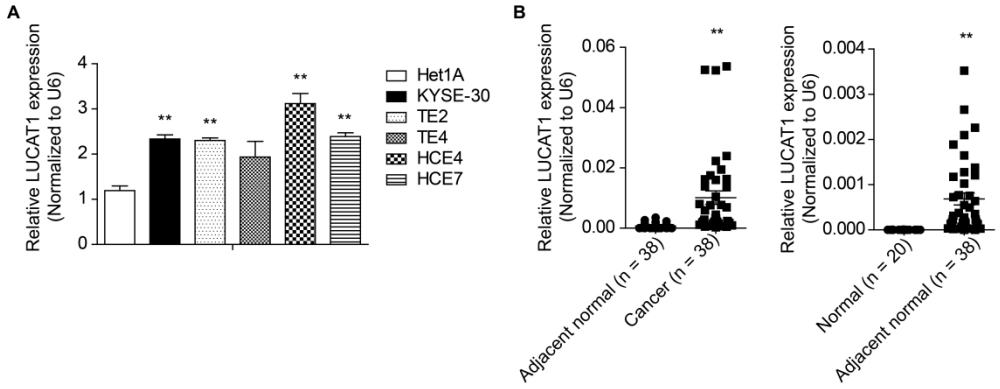

Figure 3. *LUCAT1* is overexpressed in ESCC cells and tissues. *LUCAT1* expression was increased in esophageal cancer cells KYSE-30, HCE-4, HCE-7, TE-2, and TE-3 compared to Het-1A, a normal esophageal cell (a). The expression of LUCAT1 was higher in the ESCC tissues than in the adjacent normal mucosa (n=38). The level of *LUCAT1* in the adjacent normal mucosa of ESCC patients was significantly higher than that in the normal mucosa of the normal controls (n=20) (b). The level of *LUCAT1* was measured by SYBR qRT-PCR and calculated by 2-ΔΔCT (* $p \leq 0.05$; ** $p \leq 0.01$).

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3. The expression of *LUCAT1* is correlated with prognoses of ESCC patients

Patients' clinicopathologic features were summarized in Table 1. The mean age of the patients included in the study was 68 years old, and men accounted for 81%. To analyze the effect of *LUCAT1* on the survival of ESCC, patients were categorized in terms of *LUCAT1* expression into a low group and a high group, which were determined using median values for the 24 patients whose data were available. Patients in the high group had poor survival compared to patients in the low group over an examination period of 15 months (range 1–36) (Fig. 4).

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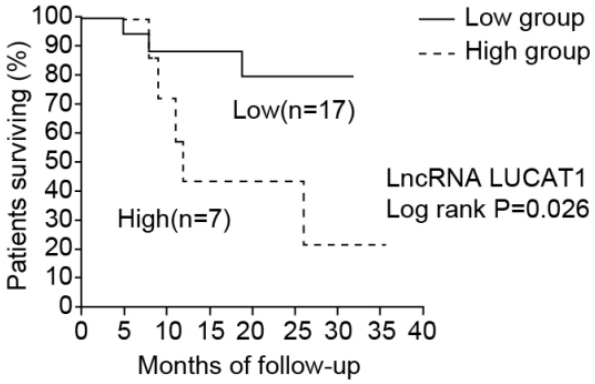

Figure 4. Overall survival among patients according to *LUCAT1* expression.

Kaplan–Meier estimates of overall survival among patients in the high and low *LUCAT1* expression groups. The mean survival of high *LUCAT1* expression-group patients was 19 months, while it was 27 months for low *LUCAT1* expression-group patients.

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4. Level of expression of *LUCAT1* determines proliferation of ESCC cells and induces apoptosis

After confirming the increased expression of *LUCAT1* in ESCC, two different siRNAs and overexpression vectors were constructed to confirm its function. Both siRNAs (siLUCAT1_1 and siLUCAT1_2) reduced the expression of the target lncRNA, *LUCAT1*, as much as 50% compared to scrambled siRNA (siCT) (Fig. 5a). The ectopic expression of LUCAT1 by the transfection of pcDNA-LUCAT1 was confirmed by qRT-PCR (Fig. 5b).

Cell proliferation assays revealed that cell proliferation significantly decreased after two different siLUCAT1s treatments in four different ESCC cell lines compared to control and siCT-treated cells. Cell proliferation, in pcDNA-LUCAT1 treatment after siLUCAT1s, was restored in comparison to siLUCAT1s treatment with statistical significance (Fig. 6). The effects of siLUCAT1s were significant in all the cell lines; however, the magnitude of the difference was larger in the KYSE-30 cell line. For this reason, the KYSE-30 cell line was used to ensure the most functional study.

To follow up on our cell proliferation results, we examined the effect of *LUCAT1* on apoptosis. When KYSE-30 cells and HCE-4 cells were treated with two different

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siLUCAT1s, the induction of apoptosis was confirmed by flow cytometry using PI/Annexin V staining (Fig. 7a). Apoptosis was increased 2 to 2.5 times in the siLUCAT1s-treated cells compared to the control group (Fig. 7b). To determine the quantitative relationship, caspase 3/7 protein was measured by the ELISA method, and it was found that siLUCAT1s significantly increased caspase 3/7 activity by 1.5 and 2 times, respectively (Fig. 7c). siLUCAT1s induced the cleavage of Poly (ADP-ribose) polymerase and caspase 9 (Fig. 7d). siLUCAT1s not only induced p53 and Bax but also reduced Bcl-2 and Bcl-XL (Fig. 7d). All these changes induced by siLUCAT1s were rescued by the ectopic expression of LUCAT1 caused by the transfection of pcDNA-LUCAT1.

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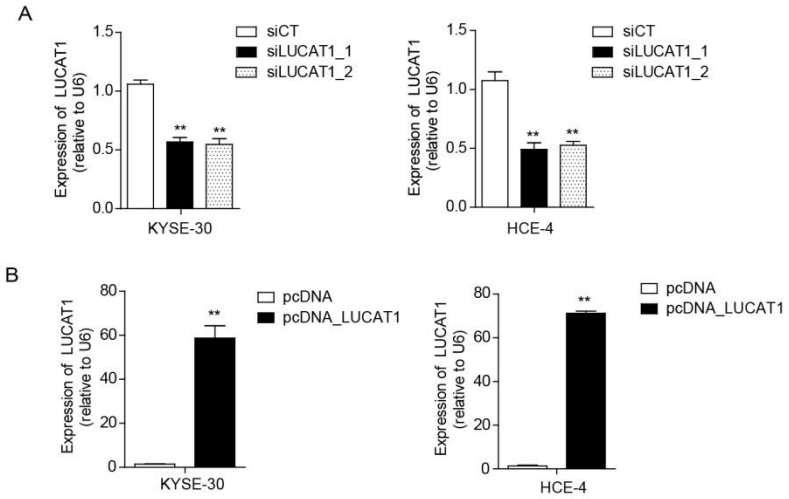

Figure 5. Generation of loss of function *LUCAT1* using siRNA and gain of function *LUCAT1* using overexpression vector in ESCC cells. *LUCAT1* expression was detected by qRT-PCR in KYSE-30 cells and HCE-4 cells transfected with (a) siRNAs and (b) pcDNA-LUCAT1. Data are the means of three independent experiments ± SEM. The asterisk represents a statistically significant difference compared with the scrambled control (* $p \leq 0.05$; ** $p \leq 0.01$).

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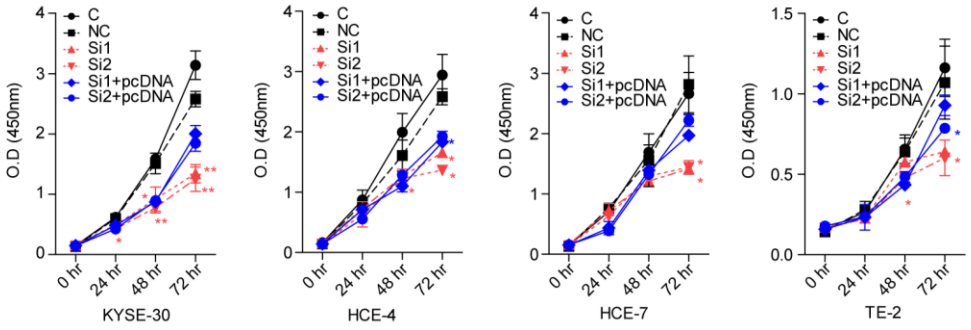

Figure 6. Inhibition of LUCAT1 expression inhibits cell proliferation of ESCC cells. KYSE-30 cells and HCE-4 cells were transfected with siLUCAT1s or pcDNA-LUCAT1, and the expression of *LUCAT1* was detected using qRT-PCR. Cell proliferation was measured by MTS assay. The data were obtained from three independent experiments. The displayed data represent the mean values ± SEM. Asterisks represent statistically significant differences when compared to pcDNA or siCT (* $p \leq 0.05$; ** $p \leq 0.01$).

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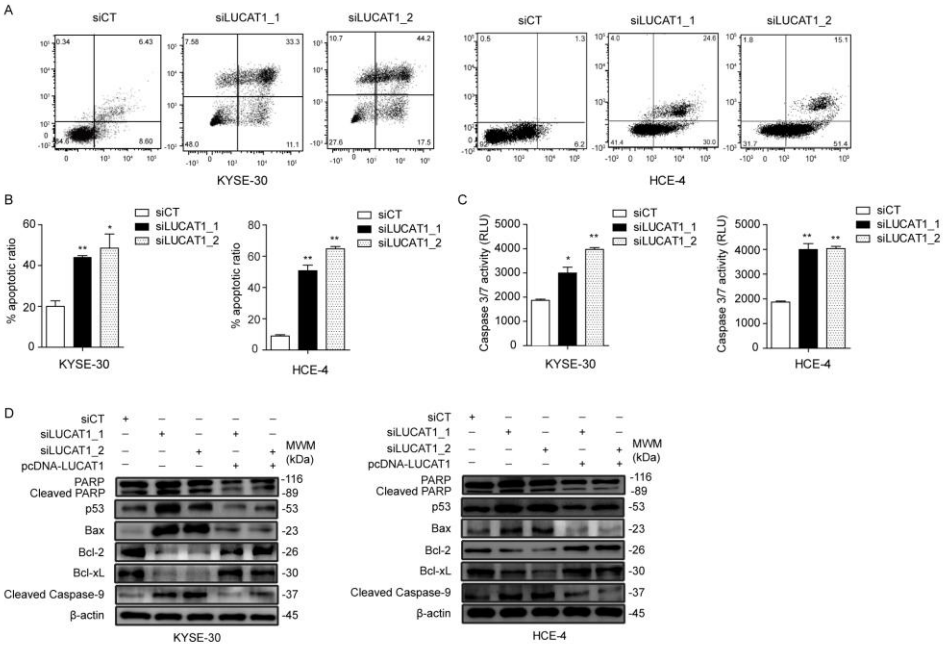

Figure 7. Inhibition of LUCAT1 expression induces apoptosis of ESCC cells. Apoptosis was measured by flow cytometry using PI/Annexin V staining (a). Early and late apoptosis combined (b). Apoptotic ratios were increased in siLUCAT1s. Caspase Glo3,7 assay was performed on KYSE-30 cells. Luminescence activity was measured using a Luminescence Microplate Reader (c). Apoptosis markers associated with *LUCAT1* expression were confirmed by immunoblot analysis on KYSE-30 cells and HCE-4 cells (d). The data were obtained from three independent experiments. The displayed data represent the mean values ± SEM. Asterisks represent statistically significant differences when compared to siCT (* $p \leq 0.05$; ** $p \leq 0.01$).

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5. Inhibition of *LUCAT1* expression inhibits invasion and migration of ESCC cells

To investigate whether *LUCAT1* is involved in the invasion and migration of ESCC, two siLUCAT1s were transfected to the KYSE-30 cells and HCE-4 cells replated onto 24 wells. Wound closure was significantly suppressed in siLUCAT1s compared to siCT (Fig. 8a). Two siRNAs for *LUCAT1* also significantly reduced the invasion of KYSE-30 cells and HCE-4 cells (Fig.8b). All the effects of siRNAs for *LUCAT1* were reduced by the transfection of pcDNA-LUCAT1 (Fig. 8a, b).

Two different siLUCAT1s increased the expression of the epithelial marker E-cadherin. On the other hand, the expression of the mesenchymal markers N-cadherin, Snail, and ZEB1 was reduced. These changes of proteins were restored by the overexpression of *LUCAT1* (Fig. 8c). These experimental results show that *LUCAT1*, which is a typical phenotype of cancer, is involved in invasion and migration.

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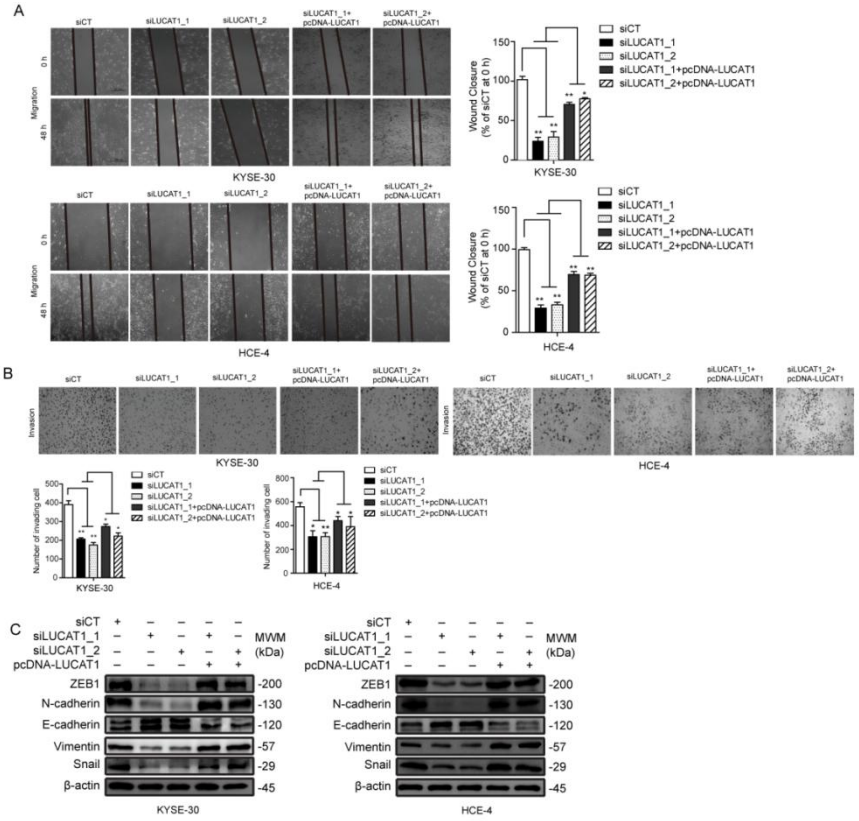

Figure 8. Inhibition of *LUCAT1* by siRNA inhibits migration and invasion of ESCC cells. The wound healing assay (a) and invasion assay (b) were performed by transfection of siCT and siLUCAT1s in KYSE-30 cells and HCE-4 cells. In the same way, EMT markers were also confirmed by immunoblot (c). Data were obtained from three independent experiments. The displayed data represent the mean values ± SEM. The asterisk indicates a statistically significant difference compared to siCT (* $p \leq 0.05$; ** $p \leq 0.01$).

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6. *LUCAT1* regulates ESCC-related genes

To investigate the mechanisms involved in the development and invasion of ESCC, a microarray of cancer-related genes was adopted after treatment of siLUCAT1s. The expression of 770 cancer-related genes was analyzed (Fig. 9a, b). We found that the expression levels of tumor suppressor genes were affected by siRNAs for *LUCAT1* (https://bioinfo.uth.edu/TSGene/index.html). qRT-PCR confirmed the findings of the microarray, and the mRNA levels of these tumor suppressor genes were significantly reduced by siLUCAT1 compared to the control. Interestingly, we found that the activities of these selected genes were regulated by methylation (Fig. 9c). Next, we performed MS-PCR after the transfection of siRNAs and found that siLUCAT1s reduced the methylation of GADD45G, GAS1, SFRP2, and HPGD. We found that siRNAs affected not only methylation status but also the activity of genes at the same time (Fig. 10a-d), confirming that *LUCAT1* is involved in the methylation of a series of genes. To determine whether *LUCAT1* is involved in the methylation of the tumor suppressor genes involved in histone modification, we observed changes in H3K4me3, H3K9me3, H3K27me3, and H3K36me3 depending on the expression of *LUCAT1* (Fig. 10e). However, we did not find significant changes in these genes after siLUCAT1 treatment.

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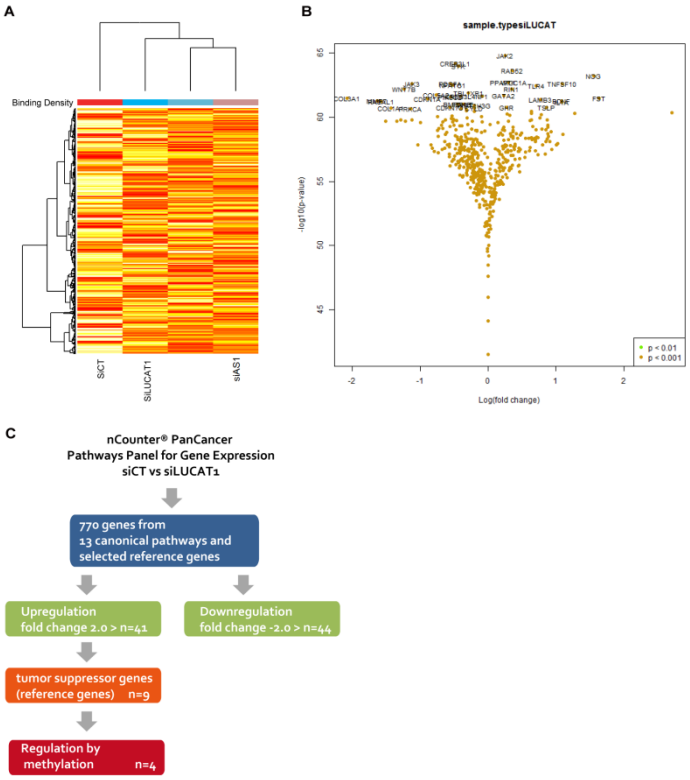

Figure 9. Cancer-related target gene expression in *LUCAT1* silencing. Heatmap of differentially expressed genes between siLUCAT1 and siCT in KYSE-30 cells (a). Volcano plot of cancer-related gene expression levels by siLUCAT1. Points to the right represent candidates that were upregulated, while points to the left represent candidates that were downregulated (b). Selection of potential target genes based on data analysis (c).

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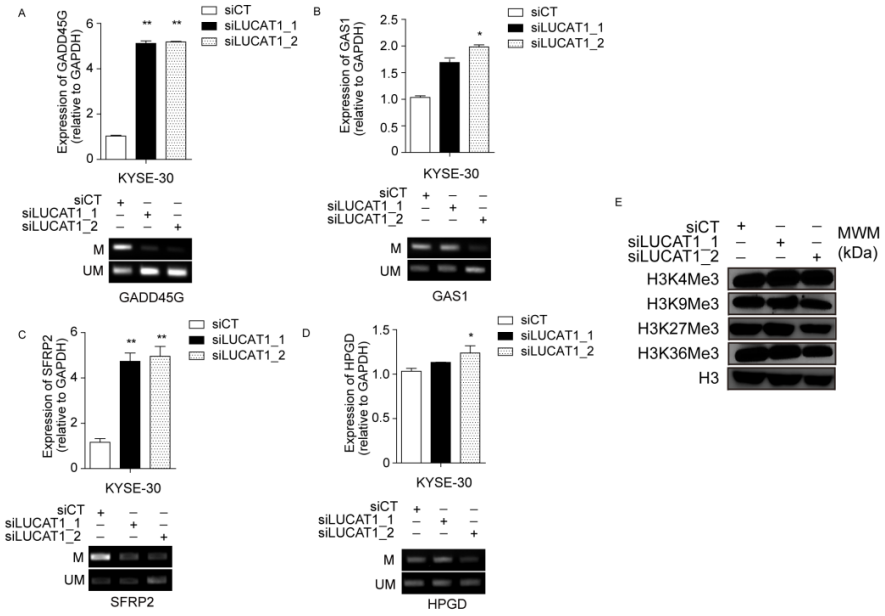

Figure 10. GADD45G, GAS1, SFRP2 and HPGD are regulated by LUCAT1 expression. Expression levels of GADD45G (a), GAS1 (b), SFRP2 (c) and HPGD (d) were confirmed by qRT-PCR using siCT or siLUCAT1 transfected into KYSE-30 cells and MS-PCR, respectively. The data were obtained from three independent experiments. The displayed data represent the mean values ± SEM. The asterisk indicates a statistically significant difference compared to siCT (* $p \leq 0.05$; ** $p \leq 0.01$). The KYSE-30 cells were transfected with siRNA targeting *LUCAT1* and subjected to immunoblots using H3K4me3, H3K9me3, H3K27me3, and H3K36me3 antibodies to measure histone levels. H3 was used as a loading control (c).

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7. *LUCAT1* regulates ubiquitination of DNMT1 by relying on UHRF1

We determined the effect of *LUCAT1* on DNMT1, which is responsible for maintenance DNA methylation and aberrant methylation patterns associated with certain human tumors and developmental abnormalities.[19] The siRNAs for *LUCAT1*s reduced the protein level of DNMT1 compared to siCT in KYSE-30 cells (Fig. 11a) but not mRNA (Fig. 11b). KYSE-30 cells were treated with cycloheximide after the knockdown of *LUCAT1*. The incubation of cells with cycloheximide did not affect the decrease of DNMT1 protein levels caused by siRNAs for *LUCAT1* (Fig. 11c). We also found that proteasome inhibitor MG132 almost completely inhibited the decrease of the DNMT1 protein levels caused by siRNAs for *LUCAT1* (Fig. 11d). Regarding this finding, we speculated that *LUCAT1* enhanced the expression of DNMT1 not at the transcriptional level but at the posttranscriptional level (e.g., methylation, phosphorylation, acetylation, and ubiquitination).

Next, we performed RIP using DNMT1 antibody and found that *LUCAT1* binds to DNMT1 (Fig. 12a). To investigate how *LUCAT1* regulates the stability of DNMT1, immunoprecipitation with DNMT1 antibody was performed after treatment with MG132 and siLUCAT1s. The ubiquitination of DNMT1 was confirmed by ubiquitination antibody at the same time. The results indicated that the ubiquitination

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of DNMT1 was more exaggerated by siLUCAT1s compared to the control (Fig. 13a). The ectopic expression of *LUCAT1* restored the reduction of DNMT1 proteins and DNMT1 ubiquitination induced by siRNAs. The exact mechanism of the ubiquitination of DNMT1 remains unclear, but it has been reported that the overexpression of ubiquitin-like-containing PHD and RING finger domains protein 1 (UHRF1) causes the ubiquitination of DNMT1.[20] To establish the relationship between *LUCAT1* and UHRF1, we introduced siRNA and an expression vector for UHRF1. The overexpression of UHRF1 alone induced the ubiquitination of DNMT1 to a similar extent observed in the knockdown of *LUCAT1* (Fig. 13b). The knockdown of UHRF1 absolutely blocked the DNMT1 ubiquitination that was induced by the siRNAs of *LUCAT1*. Thus, our results indicate that *LUCAT1* induces DNMT1 ubiquitination, which is determined by the presence of UHRF1.

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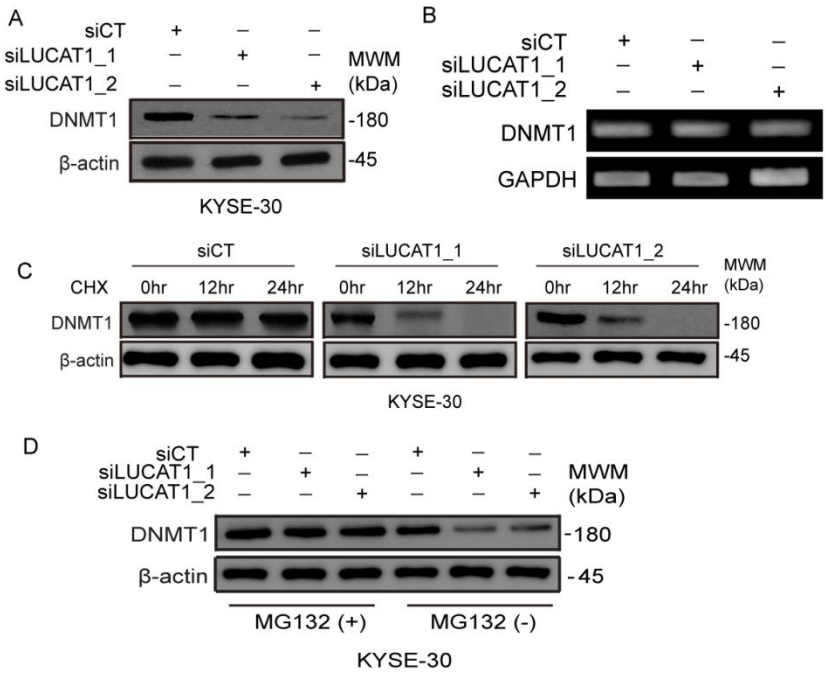

Figure 11. *LUCAT1* regulates the stability of DNMT1. KYSE-30 cells were transfected with siCT or siLUCAT1s and immunoblotted with DNMT1 antibody to confirm the expression of DNMT1 (a). RT-PCR analysis of DNMT1 expression in KYSE-30 cells transfected with siLUCAT1s (b). The expression of DNMT1 was confirmed by immunoblotting KYSE-30 cells treated with cycloheximide (100 μg/ml) (c). KYSE-30 cells transfected with siLUCAT1s were cultured in the presence or absence of MG132 and then underwent immunoblotting analysis with anti-DNMT1 (d).

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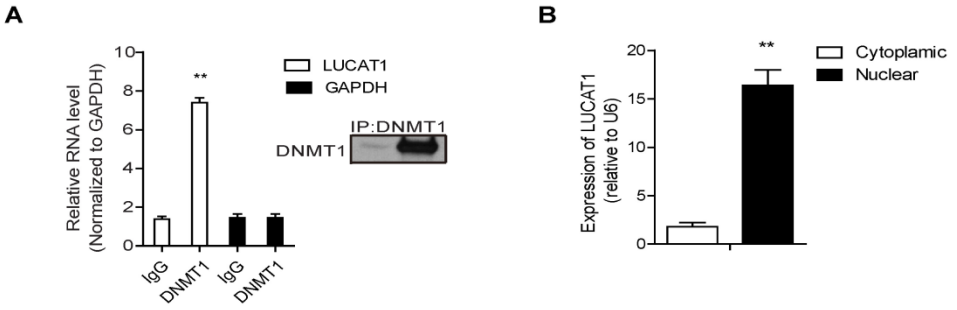

Figure 12. *LUCAT1* binding DNMT1. KYSE-30 cell lysate was immunoprecipitated with DNMT1 antibody and subjected to RIP assay to detect the binding of *LUCAT1* and DNMT1 by qRT-PCR (a). After nuclear and cytosolic separation, RNA was extracted from the nuclear and cytoplasmic fractions of KYSE-30 cells, and *LUCAT1* expression was measured by qRT-PCR (b). The data were obtained from three independent experiments. The displayed data represent the mean values ± SEM. The asterisk indicates a statistically significant difference compared to siCT (* $p \leq 0.05$; ** $p \leq 0.01$).

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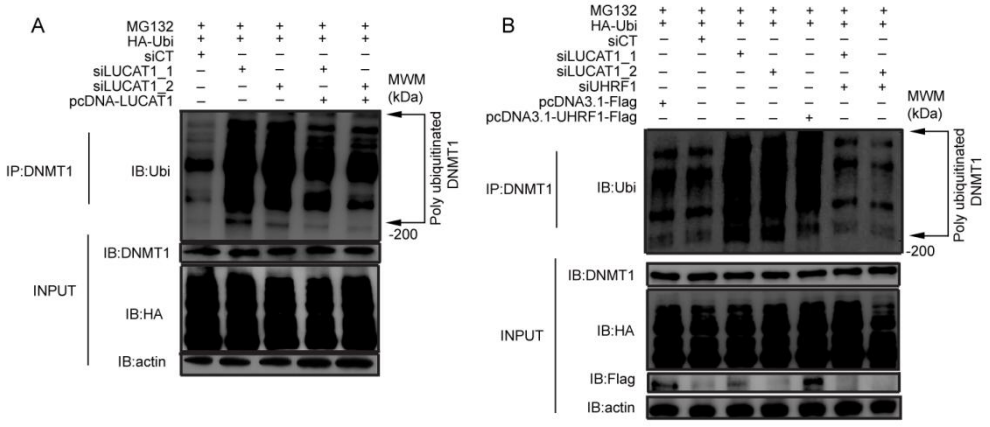

Figure 13. *LUCAT1* regulates the stability of DNMT1. KYSE-30 cells were treated with siLUCAT1s, siCT, and HA-ubiquitin transfection and MG132 (10 μM). Cell lysates were immunoprecipitated with DNMT1 antibody to detect the polyubiquitination of DNMT1 using Ub antibody (a). Flag-UHRF1 or siUHRF were transfected and detected with Ub antibody in the same manner (b).

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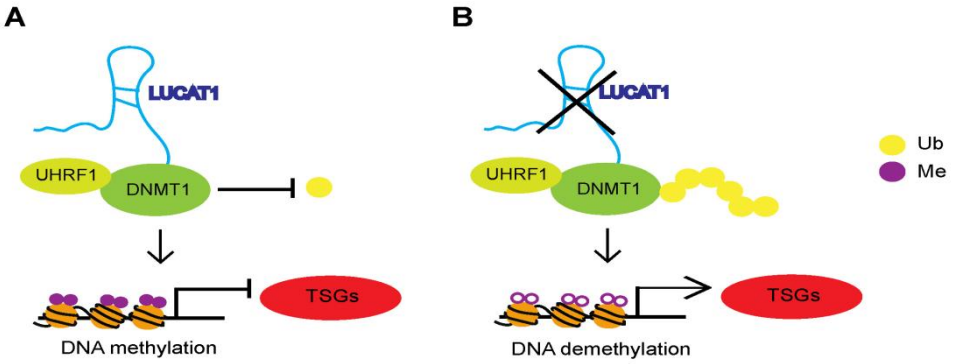

Figure 14. *LUCAT1* regulates the expression of tumor suppressor genes through regulation of DNMT1. *LUCAT1* increases the stability of DNMT1 and inhibits the expression of tumor suppressor genes. (a) When the expression of *LUCAT1* is inhibited, the ubiquitination of DNMT1 is induced and the gene expression of tumor suppressor genes is activated (b).

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Ⅳ. DISCUSSION

Long non-coding RNAs (lncRNAs) have been shown to have various roles and functions in terms of physiological and pathological aspects, and reports have shown that most non-coding RNAs have biological functions by regulating gene expression. In particular, the dysregulation of lncRNAs plays an important role in cancer formation and is closely associated with the development and progression of gastric, colon, lung, and other types of cancer. Thus, it has emerged as a biomarker for cancer diagnosis and many reports have been published.[4,21-23] Despite the many reports indicating that lncRNAs are associated with cancer, few studies have identified the specific mechanism.

Recently, the discovery and analysis of non-coding RNAs have been enhanced by RNA-seq technology, and different pipelines have been developed to identify novel lncRNAs using RNA-seq data.[5,24,25] However, despite successful studies highlighting the important roles of lncRNAs in different tissues and diseases,[12,13,15,18] little is known about the roles of lncRNAs in ESCC. Furthermore, the biological inference of lncRNA functions remains a challenging task, given their currently limited annotation status and low expression levels.

In this study, we focused on *LUCAT1*, which was highly overexpressed in ESCC

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cancer tissues according to RNA-seq data. *LUCAT1* was significantly related with the proliferation and invasion of ESCC. The survival rate of ESCC was determined by the expression of *LCUAT1*. We found that *LUCAT1* regulates the expression of DNMT1 in a UHRF1 (an epigenetic regulator of DNMT1)-dependent manner. The mechanism of action of *LUCAT1* we have found is in good agreement with the recent finding that lncRNA is directly involved in DNA methylation. In particular, *LUCAT1* directly regulated UHRF1, which acted as a key epigenetic regulator by bridging DNA methylation and chromatin modification in this study. This has not been shown in prior research and is thus a very interesting finding.

DNA methylation is one of the fundamental epigenetic mechanisms to regulate gene transcription, which involves the addition of a methyl group to cytosines that are typically restricted to CpG dinucleotides.[26] Epigenetic changes caused by DNA methylation are important for the onset and progression of cancer.[7] In particular, the promoter methylation of the tumor suppressor gene results in transcriptional silencing and loss of gene function and subsequently drives cancer development.[8] We first focused on the tumor suppressor genes whose expression is upregulated after siRNA *LUCAT1* in a microarray and subsequent qRT-PCR and whose major regulation is DNA methylation. SiLUCAT1 reduced the methylation of GADD45G, GAS1, SFRP2, and HPGD, which are known cancer tumor suppressors,[27-30] and upregulated the

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expression of these proteins. Among them, GADD45G and SFRP2 have been reported to be methylated in ESCC, and their expression has been reported to be related to cancer formation as a tumor suppressor.[31,32] We confirmed that *LUCAT1* is involved in the methylation of a series of tumor suppressor genes, and this finding was not related with histone modification.

In our study, siLUCAT1 reduced the level of DNMT1 protein in the KYSE-30 cell line but not mRNA. DNMT1 knockdown was reported to induce apoptosis, inhibit the proliferation of ESCC, and lead to the demethylation of tumor suppressor genes.[33] We found that *LUCAT1* expression was closely related with the polyubiquitination of DNMT1 in KYSE-30 through experiments on the knockdown and ectopic expression of *LUCAT1*. UHRF1 was reported as the major component to induce proteasomal degradation targeting DNMT1 by acetylation caused by acetyltransferase Tip60.[34] The overexpression of UHRF1 was reported to increase the ubiquitination of DNMT1.[20,34] We found that the knockdown of UHRF1 absolutely blocked the DNMT1 ubiquitination induced by the siRNAs of *LUCAT1*. In our study, the stability/ubiquitination of DNMT1 was regulated by *LUCAT1* in a UHRF1-dependent manner. Recently, lncRNA UPAT (ubiquitin-like plant homeodomain (PHD) and really interesting new gene (RING) finger domain-containing protein 1 (UHRF1) Protein Associated Transcript) was reported to promote colon carcinogenesis by inhibiting the

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degradation of UHRF1.[35]

To date, whether the stability of genes is regulated by lncRNAs remains unexplored. Several reports have indicated the role of lncRNA in the regulation of gene stability. Anti-differentiation ncRNA (*ANCR*) has been shown to modulate EZH2 stability, and it has been found to be a novel potential tumor suppressor in breast cancer through modulating the EMT process and cell migration.[36] Our study added one more lncRNA, *LUCAT1*, which can control DNMT1 stability by controlling ubiquitination.

LUCAT1, reported as an lncRNA *SCAL1* induced by tobacco smoke in smokers' airways and upregulated in many lung cancer cell lines, is bound to and regulated by NRF2.[17] In our study, *LUCAT1* showed a relationship with NRF2 in the ESCC cell line (data not shown), but this association was not a major pathway for the development of ESCC. Therefore, we had to find a new mechanism of *LUCAT1*'s involvement in ESCC development in this study.

LUCAT1 was significantly upregulated in ESCC tissues compared to adjacent normal tissue of patients with ESCC, and the aberrant expression of *LUCAT1* was associated with ESCC prognosis. These findings suggest that *LUCAT1* is an onset and prognostic marker of ESCC. One interesting finding in this study is that *LUCAT1* expression in adjacent normal tissues of patients with ESCC was significantly greater than in normal

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esophageal tissues. This result suggests that *LUCAT1* can be used as a predictor for the development of ESCC and that early control of *LUCAT1* can even prevent the development of ESCC.

In our study, we found that *LUCAT1* activated DNMT1, a major DNA methylation pathway, to repress the expression of tumor suppressor genes, leading to ESCC (Fig. 14). Since *LUCAT1* is expected to play a major role as a predictive marker for ESCC and a target for preventive drugs.

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Ⅴ. CONCLUSION

LUCAT1 was significantly upregulated in ESCC tissues compared with that in adjacent normal tissue of patients with ESCC. Aberrant expression of *LUCAT1* was associated with prognosis of ESCC. Thus, *LUCAT1* may be an effective marker of the onset and prognosis of ESCC.

Collectively, Our results thus suggest that *LUCAT1* regulates the stability of DNMT1 and inhibits the expression of tumor suppressors through DNA methylation, leading to the invasion and proliferation of ESCC. We identified *LUCAT1* as a potential target for drug development and as a biomarker for ESCC.

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ABSTRACT (IN KOREAN)

Long non coding RNA *LUCAT1*의 DNMT1 ubiquitination 조절을 통한 식도편평상피암의 발암기전

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식도편평상피암 (ESCC)은 예후가 좋지 않은 치명적인 악성 종양으로 암발생과 전이 기전이 잘 알려져 있지 않다. 최근에 많은 long non-coding RNA (lncRNA)가 다양한 암 발생과 전이에 관여하는 것으로 보고 되었다. 이번 연구에서는 ESCC에서 LUCAT1의 기전을 검증하였다. 13명 식도편평상피암 환자의 암 조직과 주변 정상조직의 RNA를 사용하여 RNA-seq deep sequencing을 진행하였다. lncRNA 중 *LUCAT1*은 주변 정상 조직과 비교하였을때 ESCC에서 확연하게 과발현되어 있음을 확인하였다 (p <0.001). LUCAT1은 ESCC 세포주 및 ESCC 환자의 암 조직에서 각각 정상 세포 및 주

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변 정상 조직과 비교하였을때 확연하게 과발현되어 있는 것을 확인하였다 (p <0.001). ESCC 환자의 암에 주변 정상 조직에서 *LUCAT1*의 발현은 정상 환자의 조직과 비교하여도 확연하게 높았다(p <0.001). KYSE-30 세포주와 HCE-4 세포주에서 *LUCAT1*의 발현은 apoptosis를 차단하고 KYSE-30 세포주와 HCE-4 세포주의 이동과 침습을 유도하였다. siRNA를 이용한 *LUCAT1*의 발현억제는 이러한 유전자의 메틸화를 줄임으로써 GADD45G, GAS1, HPGD 및 SFRP2를 비롯한 종양 억제 유전자들의 발현을 증가시켰다. *LUCAT1*의 발현 억제는 전사의 간섭 없이 DNA 메틸 전이 효소 1 (DNMT1)의 단백질 수준을 감소 시켰다. *LUCAT1*은 E3 ubiquitin-protein ligase UHRF1에 의존적인 방식으로 DNMT1의 ubiquitination과 분해를 조절했다. *LUCAT1*의 발현이 높은 환자는 *LUCAT1*의 발현이 낮은 환자보다 생존율이 현저히 낮았다. 결론적으로 본 연구의 결과는 lncRNA *LUCAT1*이 DNMT1의 안정성을 조절하고 ESCC의 형성과 전이로 이어지는 DNA 메틸화를 통해 종양 억제 인자의 발현을 억제함을 시사한다. 이번 연구에서는 ESCC에 대한 약물 및 바이오 마커의 표적으로 LUCAT1의 가능성을 발견했다.

핵심되는말: long non-coding RNA, *LUCAT1*, DNA 메틸 전이 효소 1,
식도 편평 상피 암